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(54) Title: TISSUE-SPECIFIC TRANSCRIPTION OF DNA SEQUENCE ENCODING A HETEROLOGOUS ENZYME FOR USE IN PRODRUG THERAPY TO LUNG CANCER

(57) Abstract

The invention relates to the use of a molecular chimaera with a prodrug in the therapy of lung cancer, the molecular chimaera comprising a transcriptional regulatory DNA sequence derived from a gene encoding a lung-associated protein or a neuroendocrine marker protein and, operatively linked to the transcriptional regulatory DNA sequence, DNA sequence encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to a lung cancer cell. The use of lung specific promoters in a GDEPT or VDEPT approach allows specific targetting of lung cancer cells.

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TISSUE-SPECIFIC TRANSCRIPTION OF DNA SEQUENCE ENCODING A HETEROLOGOUS ENZYME FOR USE IN PRODRUG THERAPY TO LUNG CANCER

The present invention relates to enzyme prodrug therapy and, in particular, to the application of this form of therapy to lung cancer.

Lung cancer is a major type of cancer in the countries of Western Europe and North America. For example, it is the most common lethal cancer in the United States. It was estimated that 172,000 people would develop lung cancer in the U.S. in 1994 and about 153,000 people would die of it. The number of deaths from lung cancer is steadily increasing. The prevalence of lung cancer in developing countries is relatively low but is expected to increase sharply with the fast spreading of tobacco smoking. It is estimated that by the year 2000, deaths related to lung cancer will increase worldwide to about 2 million, mainly as a result of an increase in cigarette smoking by young adults.

Multiple categories of cancer commonly arise in the lung. Clinically, and therapeutically, about 25% of lung cancer cases are classified as small cell lung carcinoma (SCLC). The other 75% of cases consist of squamous carcinomas, large cell carcinomas, and adenocarcinomas, and are referred to collectively as non-small cell lung carcinoma (NSCLC).

For SCLC, combination chemotherapy forms the cornerstone of therapy. Because of its relatively rapid growth rate, and its tendency to metastasize, SCLC can rarely be treated surgically. In the absence of treatment, median survival in SCLC is only a few months. After aggressive and toxic combination chemotherapy, only 10% of patients with SCLC will be alive at 2 years after diagnosis and 5% at 5 years.

For NSCLC, surgery is the major curative modality for patients without demonstrable metastatic disease. However, only a minority of cases can be cured by surgical resection. Chemotherapy and chest radiotherapy are also seldom curative. The overall 5-year survival for newly diagnosed cases of NSCLC is only 10% to 15%.

There has been no improvement in the survival rate for lung cancer patients in decades, which is a reflection both of the lack of a satisfactory screening test that could detect it in the early stages and, up to the present time, of the lack of truly

effective treatment with clear survival benefits. With the adoption in the 1970s of combination chemotherapy for SCLC, and novel surgical approaches for NSCLC, median survival improved slightly. However, very little further improvements have occurred since. For example, the 5-year survival of Americans afflicted with lung cancer improved only slightly from 12% to 13%, from 1974 through 1987 (Ries *et al.* 1991 NIH Publication 91-2789).

A therapeutic plateau has now been reached, and it is clear that new approaches are needed for lung cancer.

Gene or virus directed enzyme prodrug therapy (GDEPT or VDEPT) is potentially less toxic and more efficient as a therapy for cancer than existing therapies. GDEPT or VDEPT involves the use of a gene encoding an enzyme that is capable of converting a relatively nontoxic prodrug to its active, e.g. cytotoxic, form.

WO-A-90 07936 proposes a treatment for an infection or a hyperproliferative disorder which is characterised by the presence, in the affected cells, of a trans-acting factor capable of regulating gene expression by inserting into the cells a polynucleotide construct having a cis-acting regulatory sequence which is regulated by the trans-acting factor and an effector gene which renders said cell susceptible to protection or destruction. For example, the cis-acting region may be homologous to the HIV tar region, and the effector gene may encode ricin A or HSV-1 thymidine kinase. Upon infection with HIV, the HIV tat protein activates the tar region, and induces transcription and expression of ricin A, resulting in cell death, or of HSV-1 tk, resulting in cell death upon treatment with dideoxynucleoside agents such as acyclovir and gancyclovir.

EP-A-0 334 301 describes methods for the delivery of vectors using recombinant retrovirus wherein the vector construct directs the expression of a protein that activates a compound with little or no cytotoxicity into a toxic product in the presence of a pathogenic agent, thereby effecting localised therapy to the pathogenic agent.

EP-A-0 415 731 describes molecular chimaeras for use with prodrugs, comprising transcriptional regulatory DNA sequences capable of being selectively activated in a mammalian cell, and a DNA sequence operatively linked to the transcriptional

regulatory DNA sequence and encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to the cell. Transcriptional regulatory sequences specifically mentioned are albumin, alfafetoprotein, carcino-embryonic antigen, tyrosine hydroxylase, choline acetyl transferase, neuron-specific enolase, glial fibro acidic protein, insulin, gamma glutamyltranspeptidase, dopa decarboxylase, HER2/neu and N-myc oncogenes. Specific prodrug/enzyme combinations disclosed are purine or pyrimidine analogs/VZV tk, FC/cytosine deaminase, phenoxyacetamide derivatives of adriamycin and melphalen/penicillin V amidase, phosphate salt of etoposide, adriamycin or mitomycin C /alkaline phosphatase, para-N-bis-(2-Cl-ethyl) aminobenzylglutamic acid/carboxypeptidase G2.

According to one aspect, the present invention provides the use of a molecular chimaera for the manufacture of a medicament for use with a prodrug in the therapy of lung cancer, the molecular chimaera comprising a transcriptional regulatory DNA sequence derived from a gene encoding a lung-associated protein or a neuroendocrine marker protein and, operatively linked to the transcriptional regulatory DNA sequence, DNA sequence encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to a lung cancer cell.

According to another aspect the present invention provides a molecular chimaera for use in therapy of lung cancer with a prodrug, the molecular chimaera comprising a transcriptional regulatory DNA sequence derived from a gene encoding a lung-associated protein or a neuroendocrine marker protein and, operatively linked to the transcriptional regulatory DNA sequence, DNA sequence encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to a lung cancer cell.

The molecular chimaera of the present invention may be made utilising standard recombinant DNA techniques.

Lung is not the site for dose limiting toxicity of most anticancer agents because airway epithelial cells are well differentiated and non-dividing. Therefore, lung specific activation of prodrugs improves the selectivity of these agents. Also, the promoter elements for lung-specific genes can be used to target selectively the lung metastatic disease localized in other tissues.

The majority of small cell lung carcinomas and about 30% of non-small cell lung cancer are of neuroendocrine origin. Neuroendocrine (NE) tumors usually produce multiple markers for NE differentiation such as creatine kinase, neuron-specific enolase, L-dopa decarboxylase, chromogranin A, neural cell adhesion molecule, Leu-7, gastrin releasing peptide, synaptophysin, calcitonin, serotinin insulinoma-associated peptide and ACTH (a hormone produced from a precursor protein called proopiomelanocortin (POMC)). These peptide and amine products are used to identify the specific tumor types. NE tumor cells selectively express the genes for most of these markers because the transcriptional regulatory sequence (TRS) elements of these genes are functional only in NE tumors and in a small nest of neurons, endocrine and ganglion cells of the central and peripheral nervous systems. Therefore, the TRS elements for NE marker genes are highly specific for cancers of neuroendocrine origin.

TRS elements have been isolated and characterised for a number of NE marker genes.

The proopiomelanocortin (POMC) gene codes for the precursor of multiple peptide hormones, including ACTH, and is normally expressed only in the anterior pituitary. The sequence of the human POMC gene, including 680 base pairs preceeding the transcriptional initiation site, have been determined (Takahashi *et al.*, Nucl. Acids Res., *11*, 6847-6858 (1983)).

The chromogranin A (CgA) gene codes for an acidic glycoprotein which is involved in hormone packaging and secretion in neuroendocrine cells. The sequence of the human CgA gene including 250 base pairs preceeding the transcriptional initiation site have been determined (Mouland *et al*, J. Biol. Chem., *269*, 6918-6926 (1994)).

Gastrin-releasing peptide (GRP) is a 27-amino acid peptide hormone. Although found in neurons in the gastrointestinal tract and in the brain, highest levels of GRP are found in fetal lung and SCLC (Moody *et al.*, Science, *214*, 1246-1248 (1981)). Many small cell lung cancers overexpress GRP as well as the GRP receptor, and in these cells binding of GRP to its receptor may act as an autocrine mitogenic stimulus (Cuttitta *et al.*, Nature, *316*, 823-826 (1985)). A functional analysis of the 5-prime

flanking region of the human GRP gene has been carried out which defined a DNA fragment which conferred SCLC-specific expression to a heterologous reporter gene (Nagalla and Spindel, Cancer Res., *54*, 4461-4467 (1994)).

Proteins shown to be lung associated or lung-specific include uteroglobin or Clara cell 10 kD protein (CC10), which is a marker for lung and a marker for endometrial differentiation. It is the predominant secreted protein of lung Clara cells which line the bronchiolar epithelium. The protein is a homodimer with a molecular weight of 17 kD. In humans, detectable levels of uteroglobin is also expressed in trachea and prostate. The physiological role of the protein is not completely understood but is known to possess anti-inflammatory activity and to inhibit phospholipase A_2.

Other such proteins include pulmonary surfactant which is composed of a mixture of lipids and surfactant proteins specifically expressed in the respiratory epithelium. Their main function involves reduction in surface tension in the alveolar space and hence prevention of alveolar collapse. There are four surfactant proteins, A, B, C, and D each interacting with the lipid component differently (Weaver and Whitsett, Biochem. J., *273*, 249-264 (1991)). Of these, the regulatory element for SP-B, which direct lung specific transcription, has been identified as a 259 bp fragment (Bohinski *et al.*, J. Biol. Chem., *268*, 11160-11166 (1993) and Tami *et al.*, DNA, *8*, 75-86 (1989)).

According to the present invention, specificity of expression of the heterologous enzyme for lung cancer cells and hence selective conversion of the prodrug to the active cytotoxic form is achieved by the use of the TRS derived from a gene encoding a lung-associated or NE-marker protein. A number of specific enzyme prodrug combinations can be used in association with this lung specific TRS.

The term "heterologous enzyme" as used herein means any enzyme not present naturally in the targetted lung cancer cell. This comprises non-mammalian enzymes such as those derived from yeast or bacteria and mammalian enzymes including naturally occurring mutant mammalian enzymes or mutant mammalian enzymes which have been generated being recombinant DNA technology.

Suitable enzymes for use according to the present invention include any having a catalytic activity appropriate to the conversion of a prodrug to a therapeutically active

compound. Such enzymes include cytosine deaminase which converts the prodrug 5-fluorocytosine to toxic 5-fluorouracil, human carboxypeptidase A1 which converts the prodrug para-N-bis(2-chloroethyl)-aminobenzoyl glutamic acid into benzoic acid mustard, the enzyme alkaline phophatase which converts the prodrugs etoposidephosphate, doxorubicin phosphate and mitomycin phosphate into the corresponding toxic dephosphorylated metabolite and the enzyme penicillin-B-amidase which converts a prodrug which is a phenylacetamide derivative of doxorubicin or melphalan into its corresponding toxic metabolite.

Another preferred enzyme for use according to the present invention is β-lactamase which has particular advantages in terms of the range of toxic agents which can be presented in the form of prodrugs capable of conversion to the active agent by means of the enzyme. In principle any toxic agent can be converted to such a prodrug by conjugation with another compound through a bond capable of being cleaved by β-lactamase. According to one particularly advantageous embodiment, conjugates are formed between the toxic agent and a cephalosporin. Specific examples include conjugates of 5-fluorouracil, methotrexate and adriamycin linked in each case to a cephalosporin (see WO-A-94 01 137 and EP-A-0 382 411) and cephalosporin mustards (see EP-A-0 484 870). In each case the cephalosporin/toxic agent conjugate shows markedly reduced toxicity but can be converted to the active form by β-lactamase thus making it suitable for use as a prodrug in GDEPT. Other toxic agents can be linked to cephalosporins in a similar way.

Prodrugs for use according to the present invention may thus be based on any compound showing a suitable chemotherapeutic effect. Preferred cytotoxic compounds include nitrogen mustard agents, antifolates, nucleoside analogs, the vinca alkaloids, the anthracyclines, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, the podophyophyllotoxins, the sulfonylureas (as described in EP-A-0 222,475) and low-molecular-weight toxins such as the trichothecenes and the colchicines. Particularly examples include doxorubicin, daunorubicin, aminopterin, methotrexate, taxol, methopterin, dichloromethotrexate, mitomycin C, porfirmoycin, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, podophyllotoxin, etoposide, melphalan, vinblastine, vincristine, desacetylvinblastine hydrazide, leurosidine, vindesine, leurosine, trichothecene and desacetylcolchicine.

According to the present invention, the molecular chimaera is selectively expressed in a target lung cancer cell population. This may be taken to mean that the chimaera is expressed at a higher level in the target than in the non-target cell population and is preferably expressed predominantly or exclusively in that population. Selective expression is achieved by inclusion of the target-cell specific TRS (promoter with or without enhancer) as described above but may also be enhanced by the method of delivery of the chimaera to the target cell. Methods capable of providing target cell specific delivery of the chimaera, with subsequent stable integration and expression, include the techniques of calcium phosphate transfection, electroporation, microinjection, liposomal transfer, ballistic barrage or retroviral infection or infection using adenovirus or adeno-associated virus. For a review of this subject see Biotechniques *6(7)* (1988).

Selectivity may be obtained by a variety of such techniques. Physiologically localised delivery of the chimaera for the target cells will reduce the possibility of non-target cells expressing the chimaera. This may be achieved when for example using retroviral or liposome mediated delivery and would involve direct injection to a blood vessel known to supply the target cells. Selectivity may also be obtained using retroviral mediated chimaera delivery in the therapy of hyperproliferative disorders. Retroviruses only infect dividing cells and would therefore only introduce chimaeras to dividing cells. Liposome technology permits the delivery of the chimaera contained therein to be targetted to a particular cell type based on appropriate modifications made to the liposome coat structure

The technique of retroviral infection of cells to integrate artificial genes employs retroviral shuttle vectors which are known in the art (see for example Mol. and Cell Biol. *6*, 2895-2902 (1986)). Essentially retroviral shuttle vectors are generated using the DNA form of the retrovirus contained in a plasmid. These plasmids also contain sequences necessary for selection and growth in bacteria. Retroviral shuttle vectors are constructed using standard molecular biology techniques well known in the art. Retroviral shuttle vectors have the parental endogenous retroviral genes (e.g. gag, pol and env) removed and the DNA sequence of interest inserted, such as the molecular chimaeras which have been described. They however contain appropriate retroviral regulatory sequences for viral encapsidation, proviral insertion into the target genome, message splicing, termination and polyadenylation. Retroviral shuttle vectors have

been derived from the Moloney murine leukaemia virus (Mo-MLV) but it will be appreciated that other retroviruses can be used such as the closely related Moloney murine sarcoma virus. Certain DNA viruses may also prove to be useful as a delivery system. The bovine papilloma virus (BPV) replicates extrachromosomally so that delivery system based on BPV have the advantage that the delivered gene is maintained in a nonintegrated manner. Adenoviruses and adeno-associated viruses may also be used.

Thus according to a further aspect of the present invention there is provided a retroviral shuttle vector containing a molecular chimaera as hereinbefore defined.

The advantages of a retroviral-mediated gene transfer system are the high efficiency of the gene delivery to the targeted tissue, sequence specific integration regarding the viral genome (at the 5' and 3' long terminal repeat (LTR) sequences) and little rearrangements of delivered DNA compared to other DNA delivery systems.

Accordingly in a preferred embodiment of the present invention there is provided a retroviral shuttle vector comprising a DNA sequence comprising a 5' viral LTR sequence, a cis acting psi encapsidation sequence, a molecular chimaera as hereinbefore defined and a 3' viral LTR sequence.

In a preferred embodiment and to help eliminate non-target-specific expression of the molecular chimaera, the molecular chimaera is placed in opposite transcriptional orientation to the 5' retroviral LTR. In addition a dominant selectable marker gene may also be included which is transcriptionally driven from the 5' LTR sequence. Such a dominant selectable marker gene may be the bacterial neomycin-resistance gene NEO (aminoglycoside-3-phosphotransferase type II) which confers on eukaryotic cells resistance to the neomycin analogue G418 sulphate (Geneticin - trade mark). The NEO gene aids in the selection of packaging cells which contain these sequences.

The retroviral vector used may be based on the Moloney murine leukaemia virus but it will be appreciated that other vectors may be used. Such vectors containing a NEO gene as a selectable marker have been described, for example, the N2 vector (Science, *230*, 1395-1398 (1985)).

A theoretical problem associated with retroviral shuttle vectors is the potential of retroviral long terminal repeat (LTR) regulatory sequences transcriptionally activating a cellular oncogene at the site of integration in the host genome. This problem may be diminished by creating SIN vectors. SIN vectors are self-inactivating vectors which contain a deletion comprising the promoter and enhancer regions in the retroviral LTR. The LTR sequences of SIN vectors do not transcriptionally activate 5 or 3 genomic sequences. The transcriptional inactivation of the viral LTR sequences diminishes insertional activation of adjacent target cell DNA sequences and also aids in the selected expression of the delivered molecular chimaera SIN vectors are created by removal of approximately 299 bp in the 3 viral LTR sequence (Biotechniques, *4*, 504-512 (1986)). Thus preferably the retroviral shuttle vector of the present invention are SIN vectors.

Since the parental retroviral gag pol and env genes have been removed from these shuttle vectors a helper virus system may be utilised to provide the gag pol and env retroviral gene products trans to package or encapsidate the retroviral vector into an infective virion. This is accomplished by utilising specialised "packaging" cell lines which are capable of generating infectious synthetic virus yet are deficient in the ability to produce any detectable wild-type virus. In this way the artificial synthetic virus contains a chimaera of the present invention packaged into synthetic artificial infectious virions free of wild-type helper virus. This is based on the fact that the helper virus that is stably integrated into the packaging cell contains the viral structural genes but is lacking the psi site and cis acting regulatory sequence which must be contained in the viral genomic RNA molecule for it to be encapsidated into an infectious viral particle.

Accordingly the present invention provides an infective virion comprising a retroviral shuttle vector as hereinbefore described said vector being encapsidated within viral proteins to create an artificial infective replication-defective retrovirus.

In addition to removal of the psi site additional alterations can be made to the helper virus LTR regulatory sequences to ensure that the helper virus is not packaged in virions and is blocked at the level of reverse transcription and viral integration.

Alternatively helper virus structural genes (i.e. gag pol and env) may be individually and independently transferred into the packaging cell line. Since these viral structural genes are separated within the genome of the packaging cell, there is little chance of covert recombinations generating wild-type virus.

In a further aspect of the present invention there is provided a method for producing infective virions of the present invention by delivering the artificial retroviral shuttle vector comprising a molecular chimaera of the invention as hereinbefore described into a packaging cell line.

The packaging cell line may have stably integrated within it a helper virus lacking a psi site and other regulatory sequence as hereinbefore described or alternatively the packaging cell line may be engineered so as to contain helper virus structural genes within its genome.

The present invention further provides an infective virion as hereinbefore described for use in therapy particularly for use in the treatment of lung cancer.

The infective virion according to the invention may be formulated by techniques well known in the art and may be presented as a formulation with a pharmaceutically acceptable carrier therefor. Pharmaceutical acceptable carriers in this instance may comprise a liquid medium suitable for use as vehicles to introduce the infective virion into the patient. An example of such a carrier is saline. The infective virion may be a solution or suspension in such a vehicle. Stabilisers and antioxidants and or other excipients may also be present in such pharmaceutical formulations which may be administered to a mammal by any conventional method e.g. oral or parenteral routes. In particular the infective virion may be administered by intra-venous or intra-arterial infusion.

Accordingly the invention also provides pharmaceutical formulations comprising a molecular chimaera of the present invention contained within one of, an infective virion or a liposome or a packaging cell mix, in admixture with a pharmaceutically acceptable carrier, and pharmaceutical formulations comprising a molecular chimaera virion, vector, liposome or packaging cell mix of the present invention in admixture with a pharmaceutically acceptable carrier.

Additionally the present invention provides methods of making pharmaceutical formulations as herein described comprising mixing an artificial infective virion containing a molecular chimaera with a pharmaceutically acceptable carrier.

The invention also includes the use of any molecular chimaera, vector, virion, liposome or pharmaceutical formulation of the present invention in human therapy and in the manufacture of a medicament for use in the treatment of pathological states.

The invention also includes methods of medical therapy comprising the use of any molecular chimaera, vector, virion, liposome or pharmaceutical formulation of the present invention.

Also included within the scope of the present invention is a protein encoded by a molecular chimaera of the present invention and any combination of such a protein and a prodrug which can be catalysed by the enzyme component of that protein.

The precise dosage to be administered to a patient will ultimately be dependent upon the discretion and professional judgement of the attendant physician and will be a product of the particular targetting mechanism chosen. References contained herein to the efficiency of targetting of retroviruses, liposome etc. may be used to determine appropriate dosage levels.

The amounts and precise regime in treating a mammal, will of course depend on a number of factors including the tuype and severity of the condition to be treated. However, for carcinoma an arterial or intravenous infusion of the artificial infective virion at a titre of between 2×10^5 and 2×10^7, for example 5×10^5, 8×10^5, 2×10^6, 5×10^6 or 8×10^6, colony forming units per mil (CFU/ml) infective virions is likely to be suitable for a typical tumor. Total amount of virions infused will be dependent on tumour size and would probably be given in divided doses.

The dose of prodrug will advantageously be in the range of 0.1 to 250mg per kilogram body weight of recipient per day, preferably 0.1 to 100mg per kilogram bodyweight.

The invention is illustrated further in the following examples in which reference is made to the accompanying drawings in which:

FIG 1 shows the results of use of the POMC promoter relative to the CMV promoter in small cell lung tumours.

FIG 2 shows the results of use of the CgA promoter relative to the CMV promoter in lung tumours.

FIG 3 shows the results of expression of the human uteroglobin promoter in different human tumor lines.

FIG 4 shows expression of the surfactant protein-B promoter in different human tumour lines.

FIG 5 shows cellular location of β-lactamase activity in mammalian cells transfected with β-lactamses constructs.

EXAMPLE 1

Cloning of the POMC promoter

A 785 base pair sequence was amplified via PCR from human fibroblast genomic DNA (Clontech, Palo Alto, CA) using the following two primers: JM30;
5'-TGACAATCGCGACTGCTCTTCACAGCATCACCCTCTCCC (39-mer; SEQ ID NO 1)
and JM31;
5'-GGATCCCGGGGAAAGAGCACGGGTCC (26-mer SEQ ID NO 2).
JM30 represents POMC sequences ending at -680 of the sequence defined by Takahashi *et al.* (*supra*) with flanking sequence containing a Nru I restriction site. JM31 represents POMC sequences ending at +105 in the region containing the 5-prime untranslated portion of the POMC mRNA. The PCR reaction was carried out for 25 cycles using standard conditions and using Vent polymerase (New England Biolabs, Inc.). PCR thermal cycling conditions were 95°C, 1 min; 65°C, 3 min; 70°C, 2 min; 92°C, 1 min; 72°C, 2 min; 25 cycles then 75°C, 10 min. This PCR product was gel-purified using the Glass-Max kit (Life Technologies, Inc.) and subsequently used

for a second PCR reaction using JM30 in combination with an internal primer. The second primer consisted of the following sequence: JM32:
5'-TGACAAAAGCTTCGGCCTCTCTCGGTCGCGGCTCTTC (37-mer; SEQ ID NO 3).
JM32 represents POMC sequences ending at +22 in the region representing the 5'-untranslated portion of the POMC mRNA. PCR was carried out as above except that thermal cycling conditions were 95°C, 1 min; 92°C, 1 min; 70°C, 1 min; 72°C, 2 mins; 25 cycles then 75°C; 10 min. This PCR product was gel-purified using the Glass-Max kit (Life Technologies, Inc.), restriction digested with Nru I and Hind III (Life Technologies, Inc.) and ligated to pRc-CMV (Invitrogen, Inc) containing the secretory β-lactamase coding region (see Example 8 below). The sequence of the promoter was confirmed using the dideoxy chain-terminator sequencing method (Sanger *et al.*, 1977).

EXAMPLE 2

Cloning of the Chromogranin A Promoter

250 base pairs of sequence preceeding the transcription initiation site of the CgA promoter have been cloned. To clone the CgA promoter itself, the sequence was amplified from a human fibroblast genomic DNA preparation (Clontech, Palo Alto, CA, USA). The forward oligo used was CGN3:
5'-TGACAATCGCGACTCTTGGAAACCAGATACCCGTCGC (37-mer; SEQ ID NO 4), which contains a flanking Nru I site (TCGCGA) and sequences beginning at -211 of the human chromogranin A promoter. The reverse primer was CGN4:
5'-TGACAAAAGCTTCTCGAGCACTGCAGTGGCAGGAGC (36-mer; SEQ ID NO 5) which consisted of a flanking Hind III site and sequences beginning at +37 of the 5'-untranslated region of the chromogranin A promoter. The PCR conditions were essentially the same as were used for the POMC promoter (see Example 1 above).

EXAMPLE 3

Proopiomelanocortin Promoter

The sequence of the human POMC gene, including 680 base pairs preceeding the transcriptional initiation site, have been determined (Takahashi *et al.*, *supra*). The 680 base pair control region of the POMC promoter was fused to the β-lactamase coding region in order to utilize β-lactamase as a reporter of promoter strength.

Transient transfections were used to evaluate the expression of different promoters. Transfections were carried out by liposome-mediated DNA delivery using lipofectamine (Life Technologies, Inc., Gaithersburg, MD, USA). Experiments were performed according to manufacturer's instructions, varying the number of cells, amount of transfection reagent, and amount of DNA to determine optimum conditions. Typically, 60 x 15mm tissue culture plates containing approximately 3×10^5 to 1×10^6 cells were employed.

The POMC-β-lactamase construct was transfected into seven cell lines (Figure 1). The relative strength of the promoter was quantitated by comparing the magnitude of expression of the POMC-β-lactamase construct to expression observed in parallel transfections using a CMV promoter-β-lactamase construct. The POMC promoter displayed apparent selectivity towards small cell carcinoma lines.

EXAMPLE 4

The chromogranin A promoter

The sequence of the human CgA gene including 250 base pairs preceeding the transcriptional initiation site have been determined (Mouland *et al.*, *supra*). The strength and specificity of the promoter were evaluated as described for POMC, using the β-lactamase gene as a reporter. This promoter was found to be active in all lung lines tested (Figure 2). Expression of CgA promoter was 0.4% of CMV in a colon line (WiDr) and was 1% of CMV in an ileocoecal (HCT-8) line.

EXAMPLE 5

The Gastrin-Releasing Peptide Promoter

Many small cell lung cancers overexpress GRP as well as the GRP receptor, and in these cells binding of GRP to its receptor may act as an autocrine mitogenic stimulus (Cuttitta *et al.*, Nature, *316*, 823-826 (1985)). Recently, a functional analysis of the 5-prime flanking region of the human GRP gene has been carried out (Nagalla and Spindel, Cancer Research, *54*, 4461-4467 (1994)). A DNA fragment was defined which conferred SCLC-specific expression to a heterologous reporter gene. This sequence may be used to regulate expression of an activating enzyme gene.

EXAMPLE 6

The Uteroglobin Promoter

A 465 base pair element of the 5'-flanking region of the human uteroglobin promoter was isolated. The element was placed in front of a β-lactamase reporter gene so that the reporter was under the transcriptional control of the uteroglobin promoter. Transient transfections using lipofectamine were carried out using the CMV promoter as a control as described above. Data obtained so far (Figure 3) suggests that expression of the uteroglobin promoter is substantially restricted to non-small cell lung cancer lines.

EXAMPLE 7

Surfactant Protein B Promoter

Pulmonary surfactant is composed of a mixture of lipids and surfactant proteins. These surfactant proteins are specifically expressed in the respiratory epithelium. Their main function involves reduction in surface tension in the alveolar space and hence prevent alveolar collapse. There are four surfactant proteins, A, B, C, and D each interacting with the lipid component differently (Weaver and Whitsett, *supra*). Of these, the regulatory element for SP-B, which direct lung specific transcription, has been identified as a 259 bp fragment (Bohinski *et al.*, and Tami *et al.*, *supra*). This promoter sequence was generated using overlapping oligonucleotides in a PCR based strategy. The promoter element was tested for directing transcription of a β-lactamase reporter, *in vitro*, in various tumor lines and the results are shown in Figure 4.

EXAMPLE 8

(i) Cloning of *E. coli* β-Lactamase for Human Cell Expression

We have constructed unique DNA constructs containing the bacterial β-lactamase gene which, when delivered to human cells, result in expression of functional β-lactamase. The advantages of β-lactamase as a prodrug activating enzyme are 1) the enzyme is kinetically very efficient and 2) because of a unique activation mechanism, a prodrug of virtually any drug can be made as an efficient substrate for the enzyme. The implications of this to cancer therapy is that it permits the use of combination prodrug therapy to counter resistance phenomena as well as allows one to choose drugs appropriate to the tumor target. To target lung cancer, prodrugs of methotrexate (5798W93) and 5-fluorouracil (1614W94) have been synthesized. β-lactamase constructs have been created which give rise to secreted, intracellular and membrane-anchored forms.

(ii) Construction of Secretory β-lactamase Constructs

To create a DNA construct which would express secretory β-lactamase in human cells, the coding region of TEM β-lactamase (Sykes and Matthews, J. Antimicrob. Chemo., 2, 115-157 (1976); Ambler and Scott, Proc. Natl. Acad. Sci. USA, 75, 3732-3736 (1978)) was used. Since it exists in the periplasm of bacteria, the unmodified coding region of TEM β-lactamase contains a signal peptide (Sutcliffe, Proc. Natl. Acad. Sci. USA, 75, 3737-3741 (1978)). Sequences useful for the cloning and expression of this gene in a eukaryote were added to flanking sequence during PCR by including the sequences in the PCR primers. The sequence of the forward primer JM1 was: 5'-TTGCATAAGCTTGCCACCATGAGTATTCAACATTTCCGTGTC (42-mer; SEQ ID NO 6). The sequence of the reverse primer JM2 was: 5'-GATCTGTCTAGATTACCAATGCTTAATCAGTGAGGC (36-mer; SEQ ID NO 7). The forward primer contains a Hind III restriction site (AAGCTT) for subsequent cloning of the PCR product, and a sequence (GCCACC) which confers optimal translation effciency in vertebrates (Kozak, J. Cell Biol. *115*, 887-903 (1991)) immediately 5-prime to the initiator methionine codon (ATG) of the β-lactamase coding region. The reverse primer contains an Xba I restriction site (TCTAGA) adjacent to the stop codon (TAA) of the β-lactamase coding region.

The PCR reaction was carried out for 25 cycles using standard conditions and using Vent DNA Polymerase (New England Biolabs, Inc., Beverly, MA, USA) in 4 mM $MgSO_4$ and 200 μM of each dNTP and 1 pmol/μl forward and reverse primers. PCR thermal cycling conditions were 95°C, 1 min; 60°C, 1 min; 75°C, 1 min, 25 cycles then 75°C, 5 min. The approximately 800 base pair PCR product was gel-purified using the Glass-Max kit (Life Technologies, Inc., Gaithersburg, MD, USA). The purified PCR product was restriction digested with Hind III and Xba I, re-purified by gel electrophoresis, and ligated into the multiple cloning site of the pRc/CMV vector (InVitrogen, Inc., San Diego, CA, USA). The orientation of the β-lactamase insert in this vector places the β-lactamase gene under the transcriptional regulation of the intermediate/early CMV promoter as well as followed a bovine growth hormone poly(A) addition signal. The sequence of the construct (designated pCMV-BL) is shown in SEQ ID NO 8 along with the amino acid sequence of inserted secretory β-lactamase.

(iii) Construction of Intracellular β-lactamase Constructs

To create a DNA construct for expression of intracellular β-lactamase in human, modifications to the terminus of the β-lactamase gene in pCMV-BL were carried out using PCR. The forward primer (JM30) for these reactions consisted of the sequence:

5'-TTGCATAAGCTTGCCACCATGCACCCAGAAACGCTGGTG (39-mer; SEQ ID NO 9).

This forward primer consists of a Hind III restriction site (AAGCTT), a concensus site for optimal traslation efficiency (GCCACC) in vertebrates (Kozak, 1991 *supra*) and an ATG initiator codon immediately adjacent to the sequence representing the mature amino-terminus of TEM β-lactamase (Sutcliffe, 1978 *supra*). When used in a PCR reaction in combination with the JM2 reverse primer described above, the resulting PCR product would contain a deleted signal peptide and a new initiator methionine codon adjacent to the mature coding region of β-lactamase. This PCR reaction was carried out using PCR conditions identical to those described for pCMV-BL, except that JM30 was substituted for JM1.

The approximately 700 base pair PCR product was gel-purified using the Glass-Max kit (Life Technologies, Inc., Gaithersburg, MD, USA). The purified PCR product was restriction digested with Hind III and Xba I, repurified by gel electrophoresis, and ligated into the multiple cloning site of the pRc/CMV vector (InVitrogen, Inc., San Diego, CA, USA) as described above for pCMV-BL. The sequence of the construct (designated pCMV-ΔBL) is shown in SEQ ID NO 10 along with the amino acid sequence of inserted intracellular β-lactamase.

(iv) Construction of Membrane-Bound β-lactamase Constructs

A membrane-bound form of β-lactamase would be useful in prodrug therapies since the enzyme is active and does not diffuse from the site of expression and since the external activation of prodrug guarantees bystander effects of the activated drug. This chimeric enzyme may also have potential as a potent immunostimulatory molecule since the membrane location of the protein may enhance its presentation on MHC Class II molecules.

To create a DNA construct which would express β-lactamase inserted in the external portion of the cell membrane in human, a membrane-spanning domain was appended to the carboxy-terminus of the secretory β-lactamase coding region contained in pCMV-BL. The membrane sequence was derived from the human C mu IgM heavy

chain gene (Dorai, Nucl. Acids Res., *17*, 6412 (1989)). This was done by fusing a 300 base pair sequence representing the human IgM membrane-spanning domain (from plasmid IgM/TM/PCRII which contains exons M1 and M2 separated by a single intervening sequence) in-frame to the carboxy-terminus of the secretory β-lactamase gene.

The first step in this process was to delete the termination codon in the β-lactamase sequence contained in pCMV-BL. This was done by PCR amplification of the insert using the forward primer JM1 (see above) in combination with the reverse primer MEM1. MEM1 consists of the sequence:
5'-TGACAATCTAGAGAGGGGGAGGTGAGCGCCGACGAG (36-mer; SEQ ID NO 11).
MEM1 contains sequence representing the carboxy-terminus of secretory β-lactamase excepting the translation termination signal (TAA) which is replaced by an Xba I restriction site. The hexameric Xba I sequence is in-frame with the coding region of β-lactamase and represents a Ser-Arg amino acid sequence. This PCR product was amplified as described above, gel-purified, and cloned into the Hind III and Xba I sites of pRc-CMV. This plasmid was designated pCMV-MEM1.
To attach a carboxy-terminal membrane spanning domain, a 300 base-pair sequence from pIgM/TM/PCRII was amplified with oligos MEM2 and MEM3. MEM2 consists of the sequence:
5'-TGACAATCTAGAGAGGGGGAGGTGAGCGCCGACGAG (36-mer; SEQ ID NO 12).
MEM3 consists of the sequence:
5'-TGACAAGGGCCCCTCTGGTCTCCGATGTTCTTC (33-mer; SEQ ID NO 13).
MEM2 represents the amino-terminus of the IgM trans-membrane domain (beginning at nucleotide 489; GenBank Accession #X14939) flanked by an Xba I restriction site (TCTAGA). MEM3 represents the carboxy-terminus of the trans-membrane domain (ending at nucleotide 815; GenBank Accession #X14939) flanked by an Apa I restriction site (GGGCCC). These oligos were used to carry out PCR as described above and the approximately 300 base-pair product was restriction digested, gel-purified, and cloned into the Xba I and Apa I sites of pCMV-MEM1. The sequence of the construct (designated pCMV-BLIgM) along with the amino acid sequence of inserted membrane-anchored β-lactamase is shown in SEQ ID NO 14.

(v) Determination of Cellular Locations of Targeted β-Lactamase Protein

Confirmation of the predicted locations of each of the β-lactamase constructs was carried out using transient DNA transfections in a mammalian cell line. Transfections were carried out by liposome-mediated DNA delivery using lipofectamine (Life Technologies, Inc., Gaithersburg, MD, USA). Experiments were performed according to manufacturer's instructions, varying the number of cells, amount of transfection reagent, and amount of DNA to determine optimum conditions. Typically, 60 x 15mm tissue culture plates containing approximately 3×10^5 to 1×10^6 cells were employed. After transfections using either pCMV-BL, pCMV-dBL, or pCMV-BLIgM, transfected cells were resuspended in 50 mM Tris-Cl (pH 7.4), 0.1 mM EDTA containing PMSF and leupeptin, swollen on ice for 10 min, then lysed using a Dounce homogenizer. After centrifugation at 800 x g for 6 min, supernatant (cytosolic fraction) was recentrifuged at 30 psi for 20 minutes in a Beckman AirFuge. Pellets from both centrifugations (which include membranes and nuclei) were combined. Each fraction was assayed for activity using the chromogenic substrate PADAC, added to a final concentration of 20 mM (Calbiochem, Corp.). Absorbence at 570 nm was measured using the auto-rate assay of a Kontron Model 9310 spectrophotometer. To assess secreted β-lactamase levels, PADAC assays were carried out on the cell-free media after transfections. β-lactamase enzyme activity was measured using PADAC (-Calbiochem, Corp.) which serves as a chromogenic substrate of β-lactamase activity (Schindler and Huber, Enzyme Inhibitors, Brodbede, Ed., pp 169-176, Verlag Chemie, Weinheim (1980)). A 500 μM PADAC stock was made in water, filtered through a 0.22 μm filter, and added to media to give a final concentration of 20 μM. Decreases in absorbance at 570 nm were measured using the auto-rate assay of a Kontron UV/Vis spectrophotometer.

The data in Figure 5 show that at 48 hours after transfection with lipofectamine, large amounts of β-lactamase are secreted from cells transfected with pCMV-BL. The cellular activity seen with this construct is presumably the enzyme contained in secretory granules in the process of being exported. In contrast the activity seen using pCMV-ΔBL is completely localized to the cellular fraction. Based on the magnitude of this activity, we estimate that the enzyme from the secretory β-lactamase construct represents 5-10% of total cellular protein made per 24 hours per cell. The activity measured using the membrane construct was found almost exclusively in the membrane fractions.

In order to characterize the polarity of the active membrane form of β-lactamase, whole cell assays were carried out. Transient transfections of human lung adenocarcinoma with pCMV-BLIgM were carried out. β-Lactamase activity was detected only if the assay media was in contact with the cells, indicating that the enzyme must be membrane-bound located on the exterior face of the membrane. Activity was not detected using the same method when a stable cell line expressing the intracellular form of β-lactamase was used as a control, indicating that the substrate does not penetrate cells.

To further confirm the localization of the membrane-form of β-lactamase, stable lines were generated for use in immunohistochemistry experiments. To create stable lines, large-scale transfections in A549 cells were performed. Since pCMV-BL, pCMV-ΔBL, and pCMV-BLIgM contain the neomycin^R gene, stable lines could be selected after passaging the lines in media containing the antibiotic, G418. Clonal lines were derived which secrete β-lactamase (pCMV-BL/A549), lines which synthesize an intracellular β-lactamase (pCMV-ΔBL/A549), and lines which synthesize membrane-bound β-lactamase (pCMV-BLIgM/A549). Cells from each clone were used for immunohistochemistry using a primary rabbit anti-β-lactamase antibody followed by a fluorescein-labeled secondary goat anti-rabbit antibody. In this test, cells were not fixed prior to treating with the antibodies. Only the stable line expressing membrane-bound β-lactamase displayed fluorescent labeling above background levels (data not shown).

(vi) β-Lactamase Delivery to Cells Confers Sensitivity to Cephalosporin Prodrugs

A. β-Lactamase efficiently activates 5798W93 and 1614W94

Prodrugs of methotrexate (5798W93) and 5-fluorouracil (1614W94) represent the parent drugs linked to cephalothin. The kinetic parameters of prodrug activation were measured by incubating various concentrations of prodrug with purified β-lactamase followed by HPLC analysis to determine the rate of prodrug conversion. β-Lactamase efficiently activates both 5798W93 and 1614W94 with a k_{cat}/K_M (specificity constant) of 272 and 67 sec^{-1} mM^{-1}, respectively.

B. Combination of the β-Lactamase Gene with 5798W93 and 1614W94 Confers Toxicity

We have evaluated the *in vitro* toxicity of the β-lactamase prodrugs in the presence and absence of the β-lactamase gene. Cytotoxicity was quantitated by determining IC_{50}s in treated A549 human lung adenocarcinoma cells using an SRB-based growth inhibition assay (Nair et al., J. Med. Chem., 32, 1277-1279 (1989)).

In the absence of the β-lactamase gene, methotrexate was 10-fold more toxic than the methotrexate prodrug 5798W93, and fluorouracil was 20-fold more toxic than the fluorouracil prodrug 1614W94 (Table 1). When A549 cells which contained stable integrated copie(s) of the secretory β-lactamase gene (A549-BL) were tested, methotrexate and its prodrug 5798W93 were equally toxic (Table 1). This experiment implies that the delivery of the β-lactamase gene to tumor cells will make them sensitive to cephalosporin prodrugs.

The relatively small differential between the toxicity of methotrexate and 5-fluorouracil and their respective prodrugs in the absence of the β-lactamase gene was unexpected. This is because, for both parent drugs, the mechanism of action is well understood and the chemical modification made by attaching cephalothin to these compounds should clearly detoxify the drugs. For example, transport of methotrexate into cells depends on availability of the terminal glutamate moiety which is blocked in 5798W93. Toxicity of 5-fluorouracil depends on the availability of the N1 group since this group is necessary for glycosidic bond formation and concomitant nucleoside formation. The N1 group is blocked in 1614W94. It is clear that the observed toxicity of these prodrugs *in vitro* reflects some degree of chemical instability of the prodrugs which could result in significant breakdown of the prodrugs during the 72-hour incubation utilized in the IC_{50} determination.

Support for this notion comes from two lines of evidence. The first is that no toxicity is observed when either prodrug is given to mice at a dose equivalent to an LD_{100} for the parental drug. The lack of toxicity in these cases is explained by the relatively short half life of the drug *in vivo* ($t_{1/2} \approx 20$ minutes) in contrast to the exposure of cells to the prodrug for 72 hours *in vitro*.

The second line of evidence is shown by direct measurement of *in vitro* toxicity by short-term assays (3 hour exposure of cells to prodrug). Using a sensitive assay for cell toxicity, a 6-[^{3}H]-deoxyuridine based assay which measures inhibition of thymidylate synthase and DNA synthesis, we could measure toxicity over time as short as a three hour interval. During this shorter interval, the differential between prodrug and parent drug increased significantly (Table 2). These data are consistent with the idea that the prodrug toxicities reported in Table 1 result from chemical instability of the prodrugs over the long time-course (72 hours) of those experiments.

(vi) Antitumor Evaluation of Secretory β-Lactamase *in vivo* Using Liposome-Mediated DNA Delivery

Secretory β-lactamase and cytosine deaminase DNA constructs were compared for antitumour effects in mice bearing subcutaneous (s.c.) A549 human lung adenocarcinoma tumours. Results are shown in Table 3. Plasmid DNA expression vectors encoding either cytosine deaminase (CD) or secretory β-lactamase (BL) under the transcriptional control of the non-specific CMV promoter were encapsidated in cationic liposomes (25μg DNA; 25 nmol liposomes). Mice bearing A549 s.c. tumours were treated with five intratumoral injections of liposomal DNA. Prodrug therapy (1614W94 (50 mg/kg; i.p., qd x 5) or 5-FC (500 mg/kg; i.p., qd x 5) was initiated two days after DNA treatment. Inhibition of tumour growth was determined on day 47. Both CD and BL constructs resulted in similar antitumour activity *in vivo*. 1614W94 administration resulted in about 60% inhibition of tumour growth (Table 3). 5-FC administration resulted in about 70% inhibition of tumour growth, whereas DNA liposomes alone and 5-FU alone (25mg/kg, i.p., qd x 5) resulted in only about 20% inhibition of tumour growth (Table 3). Thus, liposomal DNA/5-FU prodrug combinations resulted in s.c. tumour regressions.

Secretory β-lactamase and cytosine deaminase DNA constructs were also evaluated by intrathoracic (i.t.) injection of liposomal DNA into the pleural space of mice bearing tumors. Results are shown in Table 4. Mice bearing human large cell lung H460 i.t. tumours received DNA encoding either CD or BL under the transcriptional control of the CMV promoter. DNA was dosed by i.t. injection on days 6, 7, 12 and 13. Prodrugs for the respective enzyme were dosed on days 7-16 (5-FC, 500 mg/kg; 1614W94, 70 mg/kg; i.p., qd x 10). Animal survival was evaluated 30 days after tumour implantation. All nontreated mice and mice treated with 5-FU (30 mg/kg i.p.,

qd x 5) died from tumour by 30 days. CMV-BL/1614W94 treatment increased survival to 60%, and CMV-CD/5-FC treatment also increased the survival to 40% (Table 4)

Table 1 Cytotoxicity (SRB Assay)

	IC_{50}	
	A549	**A549-BL**
Methotrexate 1 μM, 3h	10 nM	N.D.
5798W93 1 μM, 3h	100 nM	N.D.
5-Fluorouracil 1 μM, 5h	1.9 μM	1.4 μM
1614W94 1 μM, 5h	40 μM	1.7 μM

Table 2 Cytotoxicity (6-[^{3}H]-Deoxyuridine Assay)

	% Inhibition	
	A549	**A549-BL**
Methotrexate 1 μM, 3h	82 ± 4	88 ± 6
5798W93 1 μM, 3h	3 ± 2	38 ± 3
5-Fluorouracil 1 μM, 5h	95 ± 8	91 ± 10
1614W94 1 μM, 5h	-2 ± 3	33 ± 6

Table 3

Antitumour Effects of Secretory β-Lactamase and Cytosine Deaminase Genes in Mice Bearing Subcutaneous A549 Human Lung Adenocarcinoma

Therapy Group	Tunour Volume (mm^3)	Percentage Inhibition (Relative to Control)
Phosphate Buffered Saline (No DNA)	1268 ± 212	0
CMV-BL and 1614W94	511 ± 86	60
CMV-CD and 5-Fluorocytosine	380 ± 237	70
5-Fluorouracil alone	1021 ± 37	19

Table 4

Antitumour Effects of Secretory β-Lactamase and Cytosine Deaminase Genes in Mice Bearing Intrathoracic H460 Human Large Cell Lung Tumours

Therapy Group	Mean Days of Survival	p Value	Increased Life Span (%)
Phosphate Buffered Saline (No DNA)	20	-	-
CMV-CD + PBS	23	0.245	15
CMV-CD + 5-Fluorocytosine	27	0.009	40
CMV-BL + 1614W94	32	0.012	60

SEQ ID NO 1 = JM30 SEQ ID NO 2 = JM31 SEQ ID NO 3 = JM32
SEQ ID NO 4 = CGN3 SEQ ID NO 5 = CGN4 SEQ ID NO 6 = JM1
SEQ ID NO 7 = JM2 SEQ ID NO 8 = pCMV-BL
SEQ ID NO 9 = JM301 SEQ ID NO 10 = pCMV-ΔBL
SEQ ID NO 11 = MEM1 SEQ ID NO 12 = MEM2
SEQ ID NO 13 = MEM3 SEQ ID NO 14 = pCMV-BLIgM

SEQ ID No 8 PCMV-BL

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                CGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT

TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT

ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG CCCATTGACG

TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG

GTGGACTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT

ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG

ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG

GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT

CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC

TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG

TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAACTAGAG AACCCACTGC TTAACTGGCT

                          Hind III (683)
TATCGAAATT AATACGACTC ACTATAGGGA GACCGGAAGC TTGCCACC ATG AGT ATT
                                                  Met Ser Ile
                                                    1

CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT GCG GCA TTT TGC CTT
Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala Phe Cys Leu
      5                  10                  15

CCT GTT TTT GCT CAC CCA GAA ACG CTG GTG AAA GTA AAA GAT GCT GAA
pro val phe ala his pro glu thr leu val lys val lys asp ala glu
 20                  25                  30                  35

GAT CAG TTG GGT GCA CGA GTG GGT TAC ATC GAA CTG GAT CTC AAC AGC
Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp Leu Asn Ser
                 40                  45                  50

                                        Xmr
GGT AAG ATC CTT GAG AGT TTT CGC CCC GAA GAA CGT TTT CCA ATG ATG
Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe Pro Met Met
             55                  60                  65

AGC ACT TTT AAA GTT CTG CTA TGT GGC GCG GTA TTA TCC CGT ATT GAC
Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser Arg Ile Asp
         70                  75                  80
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GCC GGG CAA GAG CAA CTC GGT CGC CGC ATA CAC TAT TCT CAG AAT GAC
Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser Gln Asn Asp
     85                  90                  95

TTG GTT GAG TAC TCA CCA GTC ACA GAA AAG CAT CTT ACG GAT GGC ATG
Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr Asp Gly Met
100                 105                 110                 115

ACA GTA AGA GAA TTA TGC AGT GCT GCC ATA ACC ATG AGT GAT AAC ACT
Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser Asp Asn Thr
                120                 125                 130

            PvuI (1110)
GCG GCC AAC TTA CTT CTG ACA ACG ATC GGA GGA CCG AAG GAG CTA ACC
Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys Glu Leu Thr
            135                 140                 145

GCT TTT TTG CAC AAC ATG GGG GAT CAT GTA ACT CGC CTT GAT CGT TGG
Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu Asp Arg Trp
        150                 155                 160

GAA CCG GAG CTG AAT GAA GCC ATA CCA AAC GAC GAG CGT GAC ACC ACG
Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg Asp Thr Thr
    165                 170                 175

ATG CCT GTA GCA ATG GCA ACA ACG TTG CGC AAA CTA TTA ACT GGC GAA
Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu Thr Gly Glu
180                 185                 190                 195

CTA CTT ACT CTA GCT TCC CGG CAA CAA TTA ATA GAC TGG ATG GAG GCG
Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp Met Glu Ala
                200                 205                 210

GAT AAA GTT GCA GGA CCA CTT CTG CGC TCG GCC CTT CCG GCT GGC TGG
Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro Ala Gly Trp
            215                 220                 225

TTT ATT GCT GAT AAA TCT GGA GCC GGT GAG CGT GGG TCT CGC GGT ATC
Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser Arg Gly Ile
        230                 235                 240

ATT GCA GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT ATC GTA GTT ATC
Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile Val Val Ile
    245                 250                 255

TAC ACG ACG GGG AGT CAG GCA ACT ATG GAT GAA CGA AAT AGA CAG ATC
Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn Arg Gln Ile
260                 265                 270                 275

                        XbaI(1556) ApaI (1562)
GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG TAATCTAGAG GGCCCTATTC
Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
```

280 285

TATAGTGTCA CCTAAATGCT AGAGCTCGCT GATCAGCCTC GACTGTGCCT TCTAGTTGCC

AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCCTTGAC CCTGGAAGGT GCCACTCCCA

CTGTCCTTTC CTAATAAAAT GAGGAAATTG CATCGCATTG TCTGAGTAGG TGTCATTCTA

PvuII

TTCTGGGGGG TGGGGTGGGG CAGGACAGCA AGGGGGAGGA TTGGGAAGAC AATAGCAGGC

BamHI (1861)

ATGCTGGGGA TGCGGTGGGC TCTATGGAAC CAGCTGGGGC TCGAGGGGGG ATCCCCACGC

GCCCTGTAGC GGCGCATTAA GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG TGACCGCTAC

ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CCTTCCTTTC TCGCCACGTT

CGCCGGCTTT CCCCGTCAAG CTCTAAATCG GGGCATCCCT TTAGGGTTCC GATTTAGTGC

TTTACGGCAC CTCGACCCCA AAAAACTTGA TTAGGGTGAT GGTTCACGTA GTGGGCCATC

GCCCTGATAG ACGGTTTTTC GCCTTTACTG AGCACTCTTT AATAGTGGAC TCTTGTTCCA

AACTGGAACA ACACTCAACC CTATCTCGGT CTATTCTTTT GATTTATAAG ATTTCCATCG

CCATGTAAAA GTGTTACAAT TAGCATTAAA TTACTTCTTT ATATGCTACT ATTCTTTTGG

ECoRI 2321

CTTCGTTCAC GGGGTGGGTA CCGAGCTCGA ATTCTGTGGA ATGTGTGTCA GTTAGGGTGT

GGAAAGTCCC CAGGCTCCCC AGGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC

AGCAACCAGG TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA

TCTCAATTAG TCAGCAACCA TAGTCCCGCC CCTAACTCCG CCCATCCCGC CCCTAACTCC

GCCCAGTTCC GCCCATTCTC CGCCCCATGG CTGACTAATT TTTTTTATTT ATGCAGAGGC

CGAGGCCGCC TCGGCCTCTG AGCTATTCCA GAAGTAGTGA GGAGGCTTTT TTGGAGGCCT

SmaI (2671)

AGGCTTTTGC AAAAAGCTCC CGGGAGCTTG GATATCCATT TTCGGATCTG ATCAAGAGAC

AGGATGAGGA TCGTTTCGCA TGATTGAACA AGATGGATTG CACGCAGGTT CTCCGGCCGC

TTGGGTGGAG AGGCTATTCG GCTATGACTG GGCACAACAG ACAATCGGCT GCTCTGATGC

CGCCGTGTTC CGGCTGTCAG CGCAGGGGCG CCCGGTTCTT TTTGTCAAGA CCGACCTGTC

PstI (2909) PvuII (2964)

CGGTGCCCTG AATGAACTGC AGGACGAGGC AGCGCGGCTA TCGTGGCTGG CCACGACGGG

CGTTCCTTGC GCAGCTGTGC TCGACGTTGT CACTGAAGCG GGAAGGGACT GGCTGCTATT

GGGCGAAGTG CCGGGGCAGG ATCTCCTGTC ATCTCACCTT GCTCCTGCCG AGAAAGTATC

CATCATGGCT GATGCAATGC GGCGGCTGCA TACGCTTGAT CCGGCTACCT GCCCATTCGA

CCACCAAGCG AAACATCGCA TCGAGCGAGC ACGTACTCGG ATGGAAGCCG GTCTTGTCGA

TCAGGATGAT CTGGACGAAG AGCATCAGGG GCTCGCGCCA GCCGAACTGT TCGCCAGGCT

CAAGGCGCGC ATGCCCGACG GCGAGGATCT CGTCGTGACC CATGGCGATG CCTGCTTGCC

GAATATCATG GTGGAAAATG GCCGCTTTTC TGGATTCATC GACTGTGGCC GGCTGGGTGT

GGCGGACCGC TATCAGGACA TAGCGTTGGC TACCCGTGAT ATTGCTGAAG AGCTTGGCGG

CGAATGGGCT GACCGCTTCC TCGTGCTTTA CGGTATCGCC GCTCCCGATT CGCAGCGCAT

CGCCTTCTAT CGCCTTCTTG ACGAGTTCTT CTGAGCGGGA CTCTGGGGTT CGAAATGACC

GACCAAGCGA CGCCCAACCT GCCATCACGA GATTTCGATT CCACCGCCGC CTTCTATGAA

AGGTTGGGCT TCGGAATCGT TTTCCGGGAC GCCGGCTGGA TGATCCTCCA GCGCGGGGAT

CTCATGCTGG AGTTCTTCGC CCACCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA

TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA TTCTAGTTGT

BamHI (3830)

GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCCCGTCGAC CTCGAGAGCT

TGGCGTAATC ATGGTCATAG CTGTTTCCTG TGTGAAATTG TTATCCGCTC ACAATTCCAC

ACAACATACG AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC

PvuII (4029)

TCACATTAAT TGCGTTGCGC TCACTGCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC

TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCCG

CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC

ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT

GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC

ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA

ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC

CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG

CGCTTTCTCA ATGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC

TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC

GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA

GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT

ACGGCTACAC TAGAAGGACA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG

GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT

TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT

TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA

GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA AAAATGAAGT TTTAAATCAA

TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC

CTATCTCAGC GATCTGTCTA TTTCGTTCAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA

TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC

CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC AGCCGGAAGG GCCGAGCGCA

GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT TAATTGTTGC CGGGAAGCTA

GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT TGCCATTGCT ACAGGCATCG

TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA CGATCAAGGC

PvuI (5467)

GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG

TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA CTGCATAATT

ScaI (5578)

CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTAC TCAACCAAGT

CATTCTGAGA ATAGTGTATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA ATACGGGATA

XmnI(5695)

ATACCGCGCC ACATAGCAGA ACTTTAAAAG TGCTCATCAT TGGAAAACGT TCTTCGGGGC

GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC

CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA AAAACAGGAA

GGCAAAATGC CGCAAAAAAG GGAATAAGGG CGACACGGAA ATGTTGAATA CTCATACTCT

TCCTTTTTCA ATATTATTGA AGCATTTATC AGGGTTATTG TCTCATGAGC GGATACATAT

TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC CGAAAAGTGC

CACCTGACGT CGACGGATCG GGAGATCTCC CGATCCCCTA TGGTCGACTC TCAGTACAAT

CTGCTCTGAT GCCGCATAGT TAAGCCAGTA TCTGCTCCCT GCTTGTGTGT TGGAGGTCGC

TGAGTAGTGC GCGAGCAAAA TTTAAGCTAC AACAAGGCAA GGCTTGACCG ACAATTGCAT
NruI (6229)

GAAGAATCTG CTTAGGGTTA GGCGTTTTGC GCTGCTTCG

SEQ ID NO 10 - PCMV-ΔBL

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GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG      60

CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG     120

CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC     180

                                  NruI (206)
TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT     240

       SpeI (249)
GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA     300

TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC     360

CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC     420

ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT     480

NdeI (483)
ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT     540

                                           SnaBI (588)
ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA     600

TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG     660

ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC     720

AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG     780

GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA     840

                                                    Hind III (891)
CTGCTTAACT GGCTTATCGA AATTAATACG ACTCACTATA GGGAGACCGG AAGCTTGCCA     900

CC ATG CAC CCA GAA ACG CTG GTG AAA GTA AAA GAT GCT GAA GAT CAG         947
   Met His Pro Glu Thr Leu Val Lys Val Lys Asp Ala Glu Asp Gln
     1               5                  10                  15

TTG GGT GCA CGA GTG GGT TAC ATC GAA CTG GAT CTC AAC AGC GGT AAG       995
Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys
                20                  25                  30

                                  XmnI (1020)
ATC CTT GAG AGT TTT CGC CCC GAA GAA CGT TTT CCA ATG ATG AGC ACT      1043
Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe Pro Met Met Ser Thr
            35                  40                  45
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TTT AAA GTT CTG CTA TGT GGC GCG GTA TTA TCC CGT ATT GAC GCC GGG      1091
Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser Arg Ile Asp Ala Gly
        50                  55                  60

CAA GAG CAA CTC GGT CGC CGC ATA CAC TAT TCT CAG AAT GAC TTG GTT      1139
Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser Gln Asn Asp Leu Val
    65                  70                  75

 ScaI (1141)
GAG TAC TCA CCA GTC ACA GAA AAG CAT CTT ACG GAT GGC ATG ACA GTA      1187
Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr Asp Gly Met Thr Val
 80                  85                  90                  95

AGA GAA TTA TGC AGT GCT GCC ATA ACC ATG AGT GAT AAC ACT GCG GCC      1235
Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser Asp Asn Thr Ala Ala
                100                 105                 110

                      PvuI (1252)
AAC TTA CTT CTG ACA ACG ATC GGA GGA CCG AAG GAG CTA ACC GCT TTT      1283
Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe
            115                 120                 125

TTG CAC AAC ATG GGG GAT CAT GTA ACT CGC CTT GAT CGT TGG GAA CCG      1331
Leu His Asn Met Gly Asp His Val Thr Arg Leu Asp Arg Trp Glu Pro
        130                 135                 140

GAG CTG AAT GAA GCC ATA CCA AAC GAC GAG CGT GAC ACC ACG ATG CCT      1379
Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg Asp Thr Thr Met Pro
    145                 150                 155

GTA GCA ATG GCA ACA ACG TTG CGC AAA CTA TTA ACT GGC GAA CTA CTT      1427
Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu Thr Gly Glu Leu Leu
160                 165                 170                 175

ACT CTA GCT TCC CGG CAA CAA TTA ATA GAC TGG ATG GAG GCG GAT AAA      1475
Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp Met Glu Ala Asp Lys
                180                 185                 190

GTT GCA GGA CCA CTT CTG CGC TCG GCC CTT CCG GCT GGC TGG TTT ATT      1523
Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile
            195                 200                 205

GCT GAT AAA TCT GGA GCC GGT GAG CGT GGG TCT CGC GGT ATC ATT GCA      1571
Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala
        210                 215                 220

GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT ATC GTA GTT ATC TAC ACG      1619
Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile Val Val Ile Tyr Thr
    225                 230                 235

ACG GGG AGT CAG GCA ACT ATG GAT GAA CGA AAT AGA CAG ATC GCT GAG      1667
Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn Arg Gln Ile Ala Glu
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240                 245                 250                 255

                                      XbaI (1698)ApaI(1704)
ATA GGT GCC TCA CTG ATT AAG CAT TGG TAA TCTAGAGGGC CCTATTCTAT        1717
Ile Gly Ala Ser Leu Ile Lys His Trp  *
                260                 265

AGTGTCACCT AAATGCTAGA GCTCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC    1777

CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG    1837

TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC    1897

TGGGGGGTGG GGTGGGGCAG GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG    1957

                                  PvuII (1985)        Bam HI (2003)
CTGGGGATGC GGTGGGCTCT ATGGAACCAG CTGGGGCTCG AGGGGGGATC CCCACGCGCC    2017

CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA CCGCTACACT    2077

TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG CCACGTTCGC    2137

CGGCTTTCCC CGTCAAGCTC TAAATCGGGG CATCCCTTTA GGGTTCCGAT TTAGTGCTTT    2197

ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG GGCCATCGCC    2257

CTGATAGACG GTTTTTCGCC TTTACTGAGC ACTCTTTAAT AGTGGACTCT TGTTCCAAAC    2317

TGGAACAACA CTCAACCCTA TCTCGGTCTA TTCTTTTGAT TTATAAGATT TCCATCGCCA    2377

TGTAAAAGTG TTACAATTAG CATTAAATTA CTTCTTTATA TGCTACTATT CTTTTGGCTT    2437

                              EcoRI (2463)
CGTTCACGGG GTGGGTACCG AGCTCGAATT CTGTGGAATG TGTGTCAGTT AGGGTGTGGA    2497

AAGTCCCCAG GCTCCCCAGG CAGGCAGAAG TATGCAAAGC ATGCATCTCA ATTAGTCAGC    2557

AACCAGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA AGTATGCAAA GCATGCATCT    2617

CAATTAGTCA GCAACCATAG TCCCGCCCCT AACTCCGCCC ATCCCGCCCC TAACTCCGCC    2677

CAGTTCCGCC CATTCTCCGC CCCATGGCTG ACTAATTTTT TTTATTTATG CAGAGGCCGA    2737

                                                    StuI (2789)
GGCCGCCTCG GCCTCTGAGC TATTCCAGAA GTAGTGAGGA GGCTTTTTTG GAGGCCTAGG    2797

                      SmaI (2813) EcoRV (2825)
CTTTTGCAAA AAGCTCCCGG GAGCTTGGAT ATCCATTTTC GGATCTGATC AAGAGACAGG    2857

ATGAGGATCG TTTCGCATGA TTGAACAAGA TGGATTGCAC GCAGGTTCTC CGGCCGCTTG    2917

GGTGGAGAGG CTATTCGGCT ATGACTGGGC ACAACAGACA ATCGGCTGCT CTGATGCCGC    2977
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CGTGTTCCGG CTGTCAGCGC AGGGGCGCCC GGTTCTTTTT GTCAAGACCG ACCTGTCCGG    3037

                PstI (3051)
TGCCCTGAAT GAACTGCAGG ACGAGGCAGC GCGGCTATCG TGGCTGGCCA CGACGGGCGT    3097

          PvuII (3106)
TCCTTGCGCA GCTGTGCTCG ACGTTGTCAC TGAAGCGGGA AGGGACTGGC TGCTATTGGG    3157

CGAAGTGCCG GGGCAGGATC TCCTGTCATC TCACCTTGCT CCTGCCGAGA AAGTATCCAT    3217

CATGGCTGAT GCAATGCGGC GGCTGCATAC GCTTGATCCG GCTACCTGCC CATTCGACCA    3277

CCAAGCGAAA CATCGCATCG AGCGAGCACG TACTCGGATG GAAGCCGGTC TTGTCGATCA    3337

GGATGATCTG GACGAAGAGC ATCAGGGGCT CGCGCCAGCC GAACTGTTCG CCAGGCTCAA    3397

GGCGCGCATG CCCGACGGCG AGGATCTCGT CGTGACCCAT GGCGATGCCT GCTTGCCGAA    3457

TATCATGGTG GAAAATGGCC GCTTTTCTGG ATTCATCGAC TGTGGCCGGC TGGGTGTGGC    3517

GGACCGCTAT CAGGACATAG CGTTGGCTAC CCGTGATATT GCTGAAGAGC TTGGCGGCGA    3577

ATGGGCTGAC CGCTTCCTCG TGCTTTACGG TATCGCCGCT CCCGATTCGC AGCGCATCGC    3637

CTTCTATCGC CTTCTTGACG AGTTCTTCTG AGCGGGACTC TGGGGTTCGA AATGACCGAC    3697

CAAGCGACGC CCAACCTGCC ATCACGAGAT TTCGATTCCA CCGCCGCCTT CTATGAAAGG    3757

TTGGGCTTCG GAATCGTTTT CCGGGACGCC GGCTGGATGA TCCTCCAGCG CGGGGATCTC    3817

ATGCTGGAGT TCTTCGCCCA CCCCAACTTG TTTATTGCAG CTTATAATGG TTACAAATAA    3877

                                                BamHI (3972)
AGCAATAGCA TCACAAATTT CACAAATAAA GCATTTTTTT CACTGCATTC TAGTTGTGGT    3937

TTGTCCAAAC TCATCAATGT ATCTTATCAT GTCTGGATCC CGTCGACCTC GAGAGCTTGG    3997

CGTAATCATG GTCATAGCTG TTTCCTGTGT GAAATTGTTA TCCGCTCACA ATTCCACACA    4057

ACATACGAGC CGGAAGCATA AAGTGTAAAG CCTGGGGTGC CTAATGAGTG AGCTAACTCA    4117

                                                PvuII
CATTAATTGC GTTGCGCTCA CTGCCCGCTT TCCAGTCGGG AAACCTGTCG TGCCAGCTGC    4177

ATTAATGAAT CGGCCAACGC GCGGGGAGAG GCGGTTTGCG TATTGGGCGC TCTTCCGCTT    4237

CCTCGCTCAC TGACTCGCTG CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT    4297

CAAAGGCGGT AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG    4357

CAAAAGGCCA GCAAAAGGCC AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA    4417
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GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC    4477

CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG    4537

TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC    4597

TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG    4657

GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC    4717

TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA    4777

TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG    4837

GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA    4897

AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG    4957

TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT    5017

CTACGGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT    5077

TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT    5137

AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA    5197

TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA    5257

CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC    5317

GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA    5377

GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG    5437

TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG    5497

TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG    5557

                                                        PvuI (5609)
TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG    5617

TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC    5677

                                                ScaI(5720)
TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT    5737

TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA    5797

                                                XmnI (5837)
CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA    5857
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AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA 5917

ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC 5977

AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC 6037

TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TAC

SEQ ID No 14 - pCMV - BLIgm

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GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG      60

CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG     120

CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC     180

                              NruI (206)
TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT     240

        SpeI (249)
GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA     300

TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC     360

CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC     420

ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT     480

NdeI(483)
ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT     540

                                                    SnaBI (588)
ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA     600

TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG     660

ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC     720

AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG     780

GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA     840

                                                          Hind III (891)
CTGCTTAACT GGCTTATCGA AATTAATACG ACTCACTATA GGGAGACCGG AAGCTTGCCA     900

CC ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT GCG         947
   Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala
     1               5                  10                  15

GCA TTT TGC CTT CCT GTT TTT GCT CAC CCA GAA ACG CTG GTG AAA GTA       995
Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val
                 20                  25                  30

AAA GAT GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC ATC GAA CTG      1043
Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu
             35                  40                  45

                                                    XmnI (1086)
GAT CTC AAC AGC GGT AAG ATC CTT GAG AGT TTT CGC CCC GAA GAA CGT      1091
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Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg
        50                  55                  60

TTT CCA ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC GCG GTA TTA      1139
Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu
     65                  70                  75

TCC CGT ATT GAC GCC GGG CAA GAG CAA CTC GGT CGC CGC ATA CAC TAT      1187
Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr
 80                  85                  90                  95

                    ScaI (1207)
TCT CAG AAT GAC TTG GTT GAG TAC TCA CCA GTC ACA GAA AAG CAT CTT      1235
Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu
                100                 105                 110

ACG GAT GGC ATG ACA GTA AGA GAA TTA TGC AGT GCT GCC ATA ACC ATG      1283
Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met
            115                 120                 125

                        PvuI (1318)
AGT GAT AAC ACT GCG GCC AAC TTA CTT CTG ACA ACG ATC GGA GGA CCG      1331
Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro
        130                 135                 140

AAG GAG CTA ACC GCT TTT TTG CAC AAC ATG GGG GAT CAT GTA ACT CGC      1379
Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg
    145                 150                 155

CTT GAT CGT TGG GAA CCG GAG CTG AAT GAA GCC ATA CCA AAC GAC GAG      1427
Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu
160                 165                 170                 175

CGT GAC ACC ACG ATG CCT GTA GCA ATG GCA ACA ACG TTG CGC AAA CTA      1475
Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu
                180                 185                 190

TTA ACT GGC GAA CTA CTT ACT CTA GCT TCC CGG CAA CAA TTA ATA GAC      1523
Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp
            195                 200                 205

TGG ATG GAG GCG GAT AAA GTT GCA GGA CCA CTT CTG CGC TCG GCC CTT      1571
Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu
        210                 215                 220

CCG GCT GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC GGT GAG CGT GGG      1619
Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly
    225                 230                 235

TCT CGC GGT ATC ATT GCA GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT      1667
Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg
240                 245                 250                 255
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ATC GTA GTT ATC TAC ACG ACG GGG AGT CAG GCA ACT ATG GAT GAA CGA      1715
Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg
                260                 265                 270

                                                      XbaI(1761)
AAT AGA CAG ATC GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG TCT      1763
Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp Ser
            275                 280                 285

AGA GAG GGG GAG GTG AGC GCC GAC GAG GAG GGC TTT GAG AAC CTG TGG      1811
Arg Glu Gly Glu Val Ser Ala Asp Glu Glu Gly Phe Glu Asn Leu Trp
        290                 295                 300

GCC ACC GCC TCC ACC TTC ATC GTC CTC TTC CTC CTG AGC CTC TTC TAC      1859
Ala Thr Ala Ser Thr Phe Ile Val Leu Phe Leu Leu Ser Leu Phe Tyr
    305                 310                 315

AGT ACC ACC GTC ACC TTG TTC AAG GTA GCA CGG CTG TGG CAC AGG GAG      1907
Ser Thr Thr Val Thr Leu Phe Lys
320                 325

GAG GGT GCA GGG CGA GTG TGG GGC CCA GGG AGC AGC CTG GGC TGG ACG      1955


TCT AGC CCG GAG GCC CCC ACA CCA CCC CAC TGG GTC ATC TCT GCC CCG      2003


GCT CCC TTC CCG ACC ACA GGA AAG CAT TTC ACA CTG TCT CTG TTG CCT      2051


                                                      ApaI (2094)
GTA GGT GAA ATG ATC CCA ACA GAA GAA CAT CGG AGA CCA GAG G           2094


GGCCCTATTC TATAGTGTCA CCTAAATGCT AGAGCTCGCT GATCAGCCTC GACTGTGCCT    2154

TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCCGTGC CTTCCTTGAC CCTGGAAGGT    2214

GCCACTCCCA CTGTCCTTTC CTAATAAAAT GAGGAAATTG CATCGCATTG TCTGAGTAGG    2274

TGTCATTCTA TTCTGGGGGG TGGGGTGGGG CAGGACAGCA AGGGGGAGGA TTGGGAAGAC    2334

                                          PvuII (2375)   BamHI (2393)
AATAGCAGGC ATGCTGGGGA TGCGGTGGGC TCTATGGAAC CAGCTGGGGC TCGAGGGGGG    2394

ATCCCCACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG TGTGGTGGTT ACGCGCAGCG    2454
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TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT CGCTTTCTTC CCTTCCTTTC     2514

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GATTTAGTGC TTTACGGCAC CTCGACCCCA AAAAACTTGA TTAGGGTGAT GGTTCACGTA     2634

GTGGGCCATC GCCCTGATAG ACGGTTTTTC GCCTTTACTG AGCACTCTTT AATAGTGGAC     2694

TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGT CTATTCTTTT GATTTATAAG     2754

ATTTCCATCG CCATGTAAAA GTGTTACAAT TAGCATTAAA TTACTTCTTT ATATGCTACT     2814

                                        EcoRI (2853)
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GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGGCAGGCAG AAGTATGCAA AGCATGCATC     2934

TCAATTAGTC AGCAACCAGG TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC     2994

AAAGCATGCA TCTCAATTAG TCAGCAACCA TAGTCCCGCC CCTAACTCCG CCCATCCCGC     3054

CCCTAACTCC GCCCAGTTCC GCCCATTCTC CGCCCCATGG CTGACTAATT TTTTTTATTT     3114

StuI (3179)
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                                    SmaI (3203)      EcoRV
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ATCAAGAGAC AGGATGAGGA TCGTTTCGCA TGATTGAACA AGATGGATTG CACGCAGGTT     3294

CTCCGGCCGC TTGGGTGGAG AGGCTATTCG GCTATGACTG GGCACAACAG ACAATCGGCT     3354

GCTCTGATGC CGCCGTGTTC CGGCTGTCAG CGCAGGGGCG CCCGGTTCTT TTTGTCAAGA     3414

                          PstI (3441)
CCGACCTGTC CGGTGCCCTG AATGAACTGC AGGACGAGGC AGCGCGGCTA TCGTGGCTGG     3474

                        PvuII (3496)
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GGCTGCTATT GGGCGAAGTG CCGGGGCAGG ATCTCCTGTC ATCTCACCTT GCTCCTGCCG     3594

AGAAAGTATC CATCATGGCT GATGCAATGC GGCGGCTGCA TACGCTTGAT CCGGCTACCT     3654

GCCCATTCGA CCACCAAGCG AAACATCGCA TCGAGCGAGC ACGTACTCGG ATGGAAGCCG     3714

GTCTTGTCGA TCAGGATGAT CTGGACGAAG AGCATCAGGG GCTCGCGCCA GCCGAACTGT     3774

TCGCCAGGCT CAAGGCGCGC ATGCCCGACG GCGAGGATCT CGTCGTGACC CATGGCGATG     3834

CCTGCTTGCC GAATATCATG GTGGAAAATG GCCGCTTTTC TGGATTCATC GACTGTGGCC     3894
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CGCAGCGCAT CGCCTTCTAT CGCCTTCTTG ACGAGTTCTT CTGAGCGGGA CTCTGGGGTT 4074

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CTTCTATGAA AGGTTGGGCT TCGGAATCGT TTTCCGGGAC GCCGGCTGGA TGATCCTCCA 4194

GCGCGGGGAT CTCATGCTGG AGTTCTTCGC CCACCCCAAC TTGTTTATTG CAGCTTATAA 4254

TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA 4314

BamHI (4362)

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ACAATTCCAC ACAACATACG AGCCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA 4494

GTGAGCTAAC TCACATTAAT TGCGTTGCGC TCACTGCCCG CTTTCCAGTC GGGAAACCTG 4554

PvuII (4561)

TCGTGCCAGC TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGTTT GCGTATTGGG 4614

CGCTCTTCCG CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG 4674

GTATCAGCTC ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA 4734

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GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG 4854

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GGAAGCGTGG CGCTTTCTCA ATGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT 5034

CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC 5094

GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT GGCAGCAGCC 5154

ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG 5214

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GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC 5334

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ACAGGCATCG TGGTGTCACG CTCGTCGTTT GGTATGGCTT CATTCAGCTC CGGTTCCCAA 5934

CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT 5994

CCTCCGATCG TTGTCAGAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT TATGGCAGCA 6054

ScaI(6110)

CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC TGGTGAGTAC 6114

XmnI (6227)

TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCGACCGA GTTGCTCTTG CCCGGCGTCA 6174

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TCTTCGGGGC GAAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC 6294

ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC TGGGTGAGCA 6354

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GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG CACATTTCCC 6534

CGAAAAGTGC CACCTGACGT C 6555

CLAIMS:

1. Use of a molecular chimaera for the manufacture of a medicament for use with a prodrug in the therapy of lung cancer, the molecular chimaera comprising a transcriptional regulatory DNA sequence derived from a gene encoding a lung-associated protein or a neuroendocrine marker protein and, operatively linked to the transcriptional regulatory DNA sequence, a DNA sequence encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to a lung cancer cell.

2. Use according to claim 1 wherein the transcriptional regulatory DNA sequence is derived from the genes coding for proopiomelanocortin (POMC), chromogranin A, gastrin releasing peptide, uteroglobin or surfactant protein B.

3. Use according to claim 1 or 2 wherein the heterologous enzyme is β-lactamase, cytosine deaminase, thymidine kinase, penicillin amidase, alkaline phosphatase, β-glucouronidase or carboxypeptidase.

4. Use according to any of claims 1 to 3 wherein the transcriptional regulatory DNA sequence comprises a promoter.

5. Use according to claim 4 wherein the transcriptional regulatory DNA sequence also comprises an enhancer.

6. Use according to any of claims 1 to 5 wherein the prodrug is a phenylacetylated derivative of a toxic agent or a cephalosporin conjugate of a toxic agent.

7. Use according to claim 6 wherein the prodrug is a phenyl acetylated derivative of adriamycin, methotrexate or 2-aminopurine or a cephalosporin conjugate of 5-fluorouracil, methotrexate or adriamycin.

8. A molecular chimaera for use in therapy of lung cancer with a prodrug, the molecular chimaera comprising a transcriptional regulatory DNA sequence derived from a gene encoding a lung-associated protein or a neuroendocrine marker protein and, operatively linked to the transcriptional regulatory DNA sequence, DNA sequence

encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to a lung cancer cell.

9. A molecular chimaera according to claim 8 wherein the transcriptional regulatory DNA sequence is derived from the genes coding for proopiomelanocortin (POMC), chromogranin A, gastrin releasing peptide, uteroglobin or surfactant protein B.

10. A molecular chimaera according to claim 8 or 9 wherein the heterologous enzyme is β-lactamase, cytosine deaminase, thymidine kinase, penicillin amidase, alkaline phosphatase, β-glucouronidase or carboxypeptidase.

11. A molecular chimaera according to any of claims 8 to 10 wherein the transcriptional regulatory DNA sequence comprises a promoter.

12. A molecular chimaera according to claim 11 wherein the transcriptional regulatory DNA sequence also comprises an enhancer.

13. A vector containing a molecular chimaera according to any of claims 8 to 12.

14. A vector according to claim 13 which is a viral vector.

15. A vector according to claim 14 which is a retroviral vector.

16. A packaging cell line containing a viral vector according to claim 14 or 15.

17. An infective virion generated from a packaging cell line according to claim 16.

18. An infective virion according to claim 17 which is a retrovirus.

19. A liposome containing a molecular chimaera according to any of claims 8 to 12 or a vector according to any of claims 13 to 15.

20. Use of a vector according to any of claims 13 to 15, a packaging cell line according to claim 16, an infective virion according to claim 17 or 18 or a liposome

according to claim 19 for the manufacture of a medicament for use with a prodrug in the therapy of lung cancer.

21. Use according to any of claim 20 wherein the prodrug is a phenylacetylated derivative of a toxic agent or a cephalosporin conjugate of a toxic agent.

22. Use according to claim 21 wherein the prodrug is a phenyl acetylated derivative of adriamycin, methotrexate or 2-aminopurine or a cephalosporin conjugate of 5-fluorouracil, methotrexate or adriamycin.

23. A pharmaceutical formulation suitable for use in the therapy of lung cancer with a prodrug, the formulation comprising a molecular chimaera according to any of claims 8 to 12, a vector according to any of claims 13 to 15, a packaging cell line according to claim 16, an infective virion according to claim 17 or 18 or a liposome according to claim 19 together with a pharmaceutically acceptable carrier

24. Use according to any of claims 1 to 7 or 20 to 22 wherein they therapy comprises administration of the medicament by calcium phosphate transfection, electroporation, microinjection, liposomal transfer, ballistic barrage of retroviral infection.

25. Use according to claim 24 wherein the therapy comprises administration of the medicament by liposomal transfer.

26. Use according to claim 25 wherein the therapy comprises administration of the medicament by liposomal transfer using a bronchioscope.

27. Method of treating cancer which comprises administering to a mammal an effective amount of chimeara as claimed in any one of claims 8 to 12, a vector as claimed in any one of claims 13 to 15, a packaging cell line as claimed in claim 16, an infective virion as claimed in claim 17 or 18 or a liposome according to claim 19.

FIG. 1.

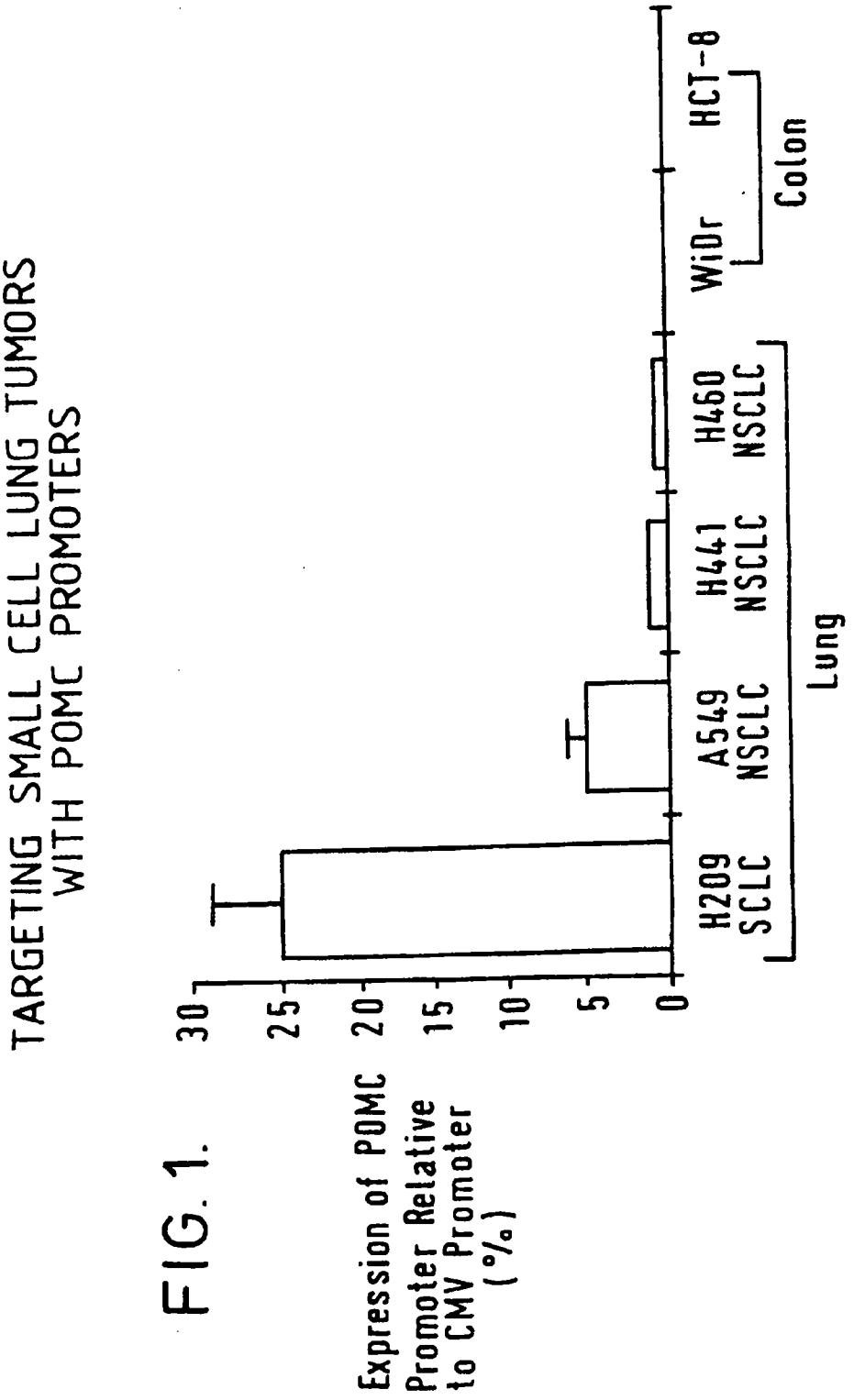
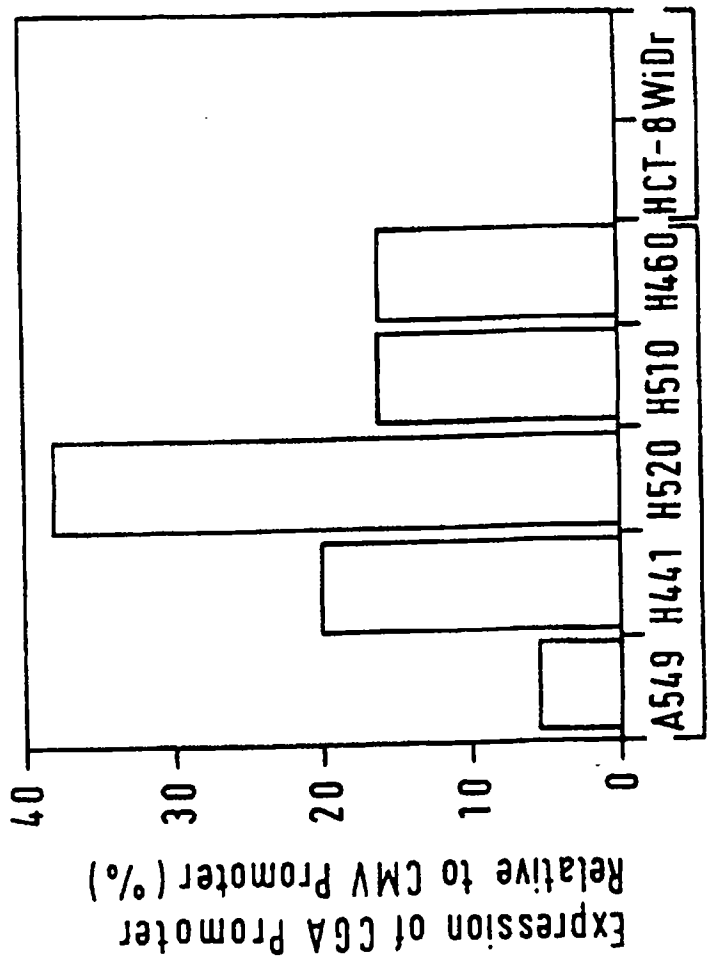

TARGETING SMALL CELL LUNG TUMORS
WITH POMC PROMOTERS
Expression of POMC
Promoter Relative
to CMV Promoter
(%)
30
25
20
15
10
5
0
H209
SCLC
A549
NSCLC
H441
NSCLC
H460
NSCLC
Lung
WiDr
HCT-8
Colon

FIG. 2.

SPECIFICITY OF CGA: A LUNG TUMOR SPECIFIC PROMOTER



CGA = CHROMOGRANIN A

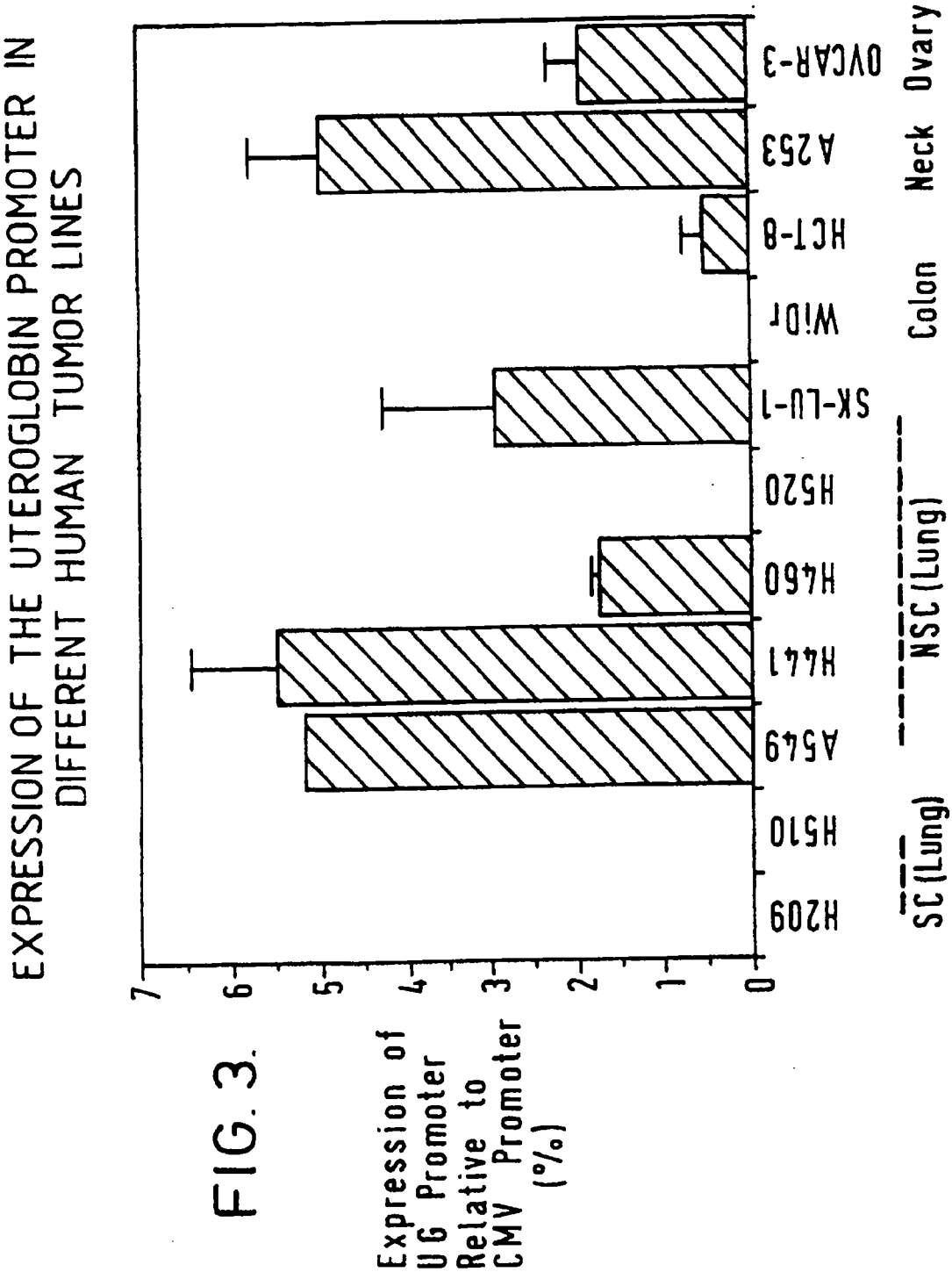

EXPRESSION OF THE UTEROGLOBIN PROMOTER IN DIFFERENT HUMAN TUMOR LINES
Expression of UG Promoter Relative to CMV Promoter (%)
0
1
2
3
4
5
6
7
H209
H510
A549
H441
H460
H520
SK-LU-1
WiDr
HCT-8
A253
OVCAR-3
SC (Lung)
NSC (Lung)
Colon
Neck
Ovary

FIG. 3.

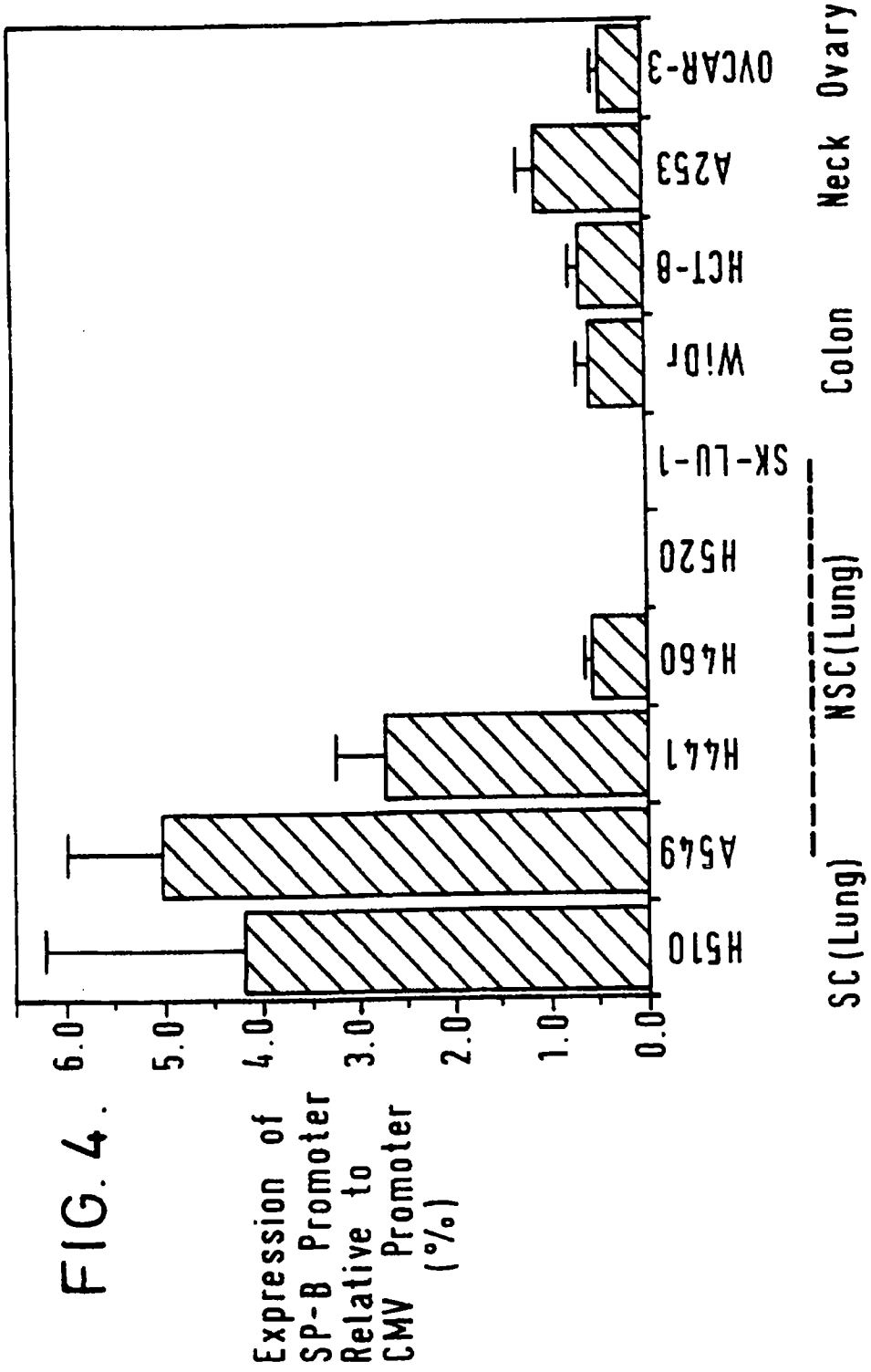
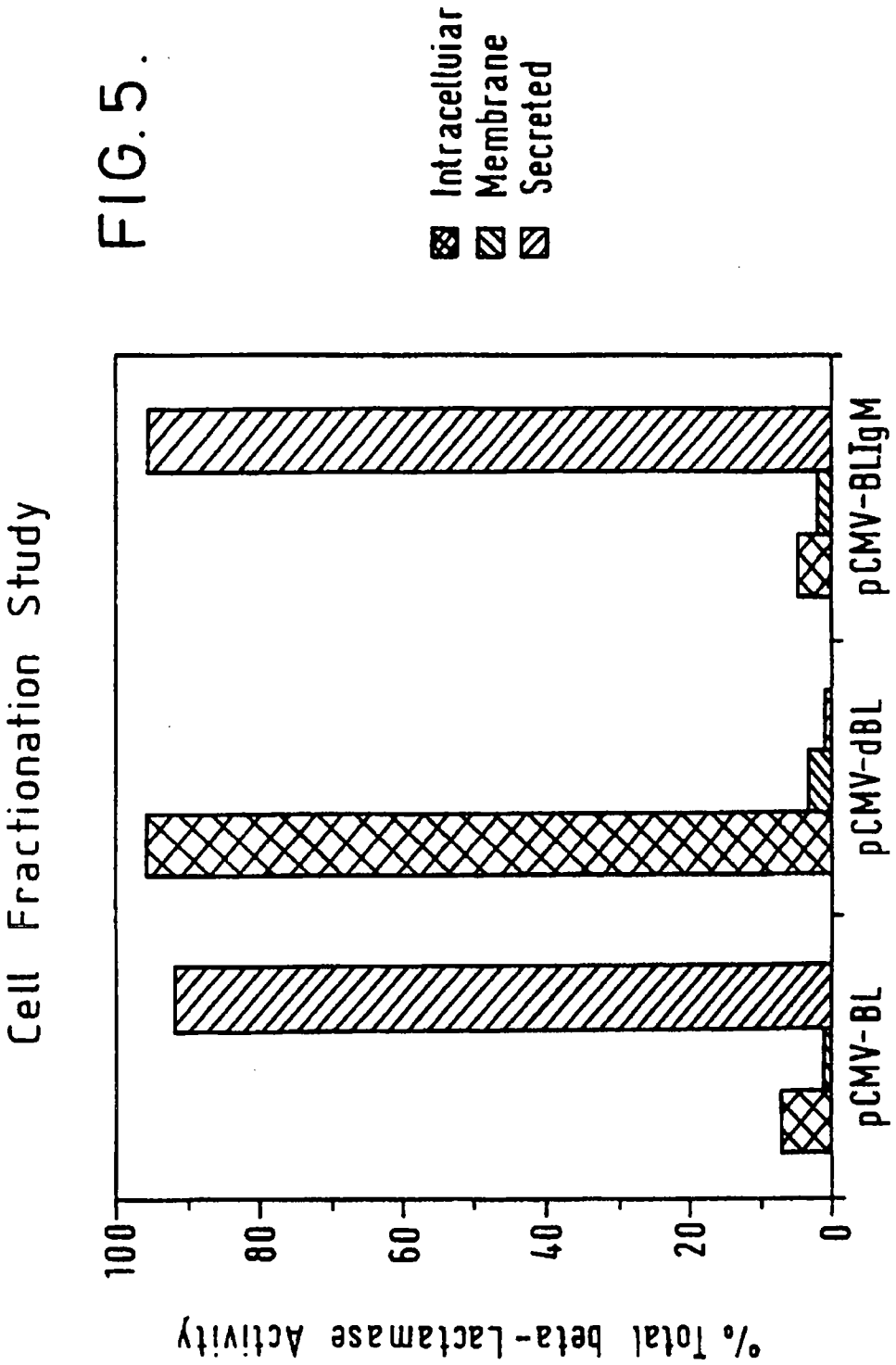

EXPRESSION OF THE SURFACTANT PROTEIN-B PROMOTER IN DIFFERENT HUMAN TUMOR LINES
Expression of SP-B Promoter Relative to CMV Promoter (%)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
H510
A549
H441
H460
H520
SK-LU-1
WiDr
HCT-8
A253
OVCAR-3
SC(Lung)
NSC(Lung)
Colon
Neck
Ovary

FIG. 4.

FIG. 5.


Intracelluiar
Membrane
Secreted
Cell Fractionation Study
% Total beta-Lactamase Activity
100
80
60
40
20
0
pCMV-BL
pCMV-dBL
pCMV-BLIgM